UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

Note to Reader

Background: As part of its effort to involve the public in the implementation of the Food Quality Protection Act of 1996 (FQPA), which is designed to ensure that the United States continues to have the safest and most abundant food supply. EPA is undertaking an effort to open public dockets on the organophosphate pesticides. These dockets will make available to all interested parties documents that were developed as part of the U.S. Environmental Protection Agency's process for making reregistration eligibility decisions and tolerance reassessments consistent with FQPA. The dockets include preliminary health assessments and, where available, ecological risk assessments conducted by EPA, rebuttals or corrections to the risk assessments submitted by chemical registrants, and the Agency's response to the registrants' submissions.

The analyses contained in this docket are preliminary in nature and represent the information available to EPA at the time they were prepared. Additional information may have been submitted to EPA which has not yet been incorporated into these analyses, and registrants or others may be developing relevant information. It's common and appropriate that new information and analyses will be used to revise and refine the evaluations contained in these dockets to make them more comprehensive and realistic. The Agency cautions against premature conclusions based on these preliminary assessments and against any use of information contained in these documents out of their full context. Throughout this process, If unacceptable risks are identified, EPA will act to reduce or eliminate the risks.

There is a 60 day comment period in which the public and all interested parties are invited to submit comments on the information in this docket. Comments should directly relate to this organophosphate and to the information and issues available in the information docket. Once the comment period closes, EPA will review all comments and revise the risk assessments, as necessary.

These preliminary risk assessments represent an early stage in the process by which EPA is evaluating the regulatory requirements applicable to existing pesticides. Through this opportunity for notice and comment, the Agency hopes to advance the openness and scientific soundness underpinning its decisions. This process is designed to assure that America continues to enjoy the safest and most abundant food supply. Through implementation of EPA's tolerance reassessment program under the Food Quality Protection Act, the food supply will become even safer. Leading health experts recommend that all people eat a wide variety of foods, including at least five servings of fruits and vegetables a day.

Note: This sheet is provided to help the reader understand how refined and developed the pesticide file is as of the date prepared, what if any changes have occurred recently, and what new information, if any, is expected to be included in the analysis before decisions are made. **It is not meant to be a summary of all current information regarding the chemical.** Rather, the sheet provides some context to better understand the substantive material in the docket (RED chapters, registrant rebuttals, Agency responses to rebuttals, etc.) for this pesticide.

Further, in some cases, differences may be noted between the RED chapters and the Agency's comprehensive reports on the hazard identification information and safety factors for all organophosphates. In these cases, information in the comprehensive reports is the most current and will, barring the submission of more data that the Agency finds useful, be used in the risk assessments.

Jack E. Housenger, Acting Director
Special Review and Reregistration Division

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PREVENTION, PESTICIDES, AND TOXIC SUBSTANCES
WASHINGTON, D.C. 20460

September 15, 1999

MEMORANDUM

SUBJECT: PHOSALONE. Toxicology Chapter for the Reregistration Eligibility Decision.

TO: Deanna Scher, Chemical Review Manager
Reregistration Branch 1
Special Review and Reregistration Division

FROM: Kit Farwell, D.V.M., Toxicologist
Reregistration Branch 1
Health Effects Division (7509C)

THRU: Whang Phang, Ph.D., Senior Scientist
Reregistration Branch 1
Health Effects Division (7509C)

DP Barcode: D256366 Submission: S562137
PC Code: 097701 Tox Chem No: 660A

BACKGROUND: The toxicology database for phosalone has been reviewed for a Reregistration Eligibility Decision (RED) in support of an import tolerance. The relevant data on phosalone were also evaluated by the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) on July 22, 1999 and by the HED Food Quality Protection Act (FQPA) Committee on August 16, 1999.

The HIARC requested that an unscheduled DNA synthesis assay be repeated. Additional data is needed to upgrade a metabolism study. However, the currently available toxicological data base on Phosalone is adequate to support a Reregistration Eligibility Decision for an import tolerance. A summary of the toxicology database and conclusions of the HIARC and FQPA Committee are included in the following toxicology chapter.

PHOSALONE TOXICOLOGY CHAPTER

TABLE OF CONTENTS

I. TOXICOLOGY DATA BASE 1
A. Hazard Profile 1
B. Acute Toxicity 1
Table 1. Acute Toxicity Profile of Phosalone. 2
C. Subchronic Toxicity 2
D. Chronic Toxicity 2
Chronic Dog Study 2
Combined Carcinogenicity/Toxicity Study in Rats 4
E. Carcinogenicity 5
Combined Carcinogenicity/Toxicity Study in Rats 5
Mouse Carcinogenicity Study 5
F. Developmental Toxicity 6
Developmental Toxicity in Rats 6
Developmental Toxicity in Rabbits 6
G. Reproductive Toxicity 7
Reproductive Toxicity in Rats 7
H. Mutagenicity Studies 8
Cytogenetic Assay 8
Bacterial Mutagenicity Assay 8
Unscheduled DNA synthesis assay 9
I. Metabolism Study 10
Metabolism Study in Rats 10
J. Neurotoxicity Studies 11
Acute Neurotoxicity Study in Rats 11
Acute Rangefinding Neurotoxicity Study 12
Time of Peak Effect Neurotoxicity Study 13
Subchronic Neurotoxicity 13

II. FOOD QUALITY PROTECTION ACT ISSUES 14
A. Susceptibility Issues 14
B. Safety Factor Recommendation and Rationale 14

III. TOXICITY ENDPOINT SELECTION 15
TABLE 2. Toxicological endpoints for use in human risk assessment 16

IV. DATA GAPS 16

I. TOXICOLOGY DATA BASE

A. Hazard Profile

The toxicological data base on Phosalone is adequate to support a Reregistration Eligibility Decision for an import tolerance.

Phosalone is an insecticide and acaricide whose toxicity is due to inhibition of acetylcholinesterase enzyme. Phosalone is in acute toxicity category 2 by the oral route. The main toxic effects seen in the subchronic and chronic studies were inhibition of plasma, red blood cell, and brain cholinesterase, and associated clinical signs due to the cholinesterase inhibition. No adverse effect levels (NOAELs) for cholinesterase inhibition were not determined in some studies because cholinesterase activity was still inhibited at the lowest dose, but this did not interfere with toxicity endpoint selection.

There was no increased susceptibility to offspring from *in utero* or post natal exposure to phosalone. Toxicity in the developmental rat and rabbit studies included increased post-implantation loss; toxicity in the rat reproduction study included body weight decrements and pup mortality. The Food Quality Protection Act (FQPA) Safety Factor Committee recommended that the **10x FQPA safety factor** for protection of infants and children be **reduced to 1x**. A developmental neurotoxicity study was not initially required under current criteria, however, EPA plans to publish a Federal Register Notice shortly which will require that registrants of "import tolerance" organophosphates, including phosalone, submit acute, subchronic, and developmental neurotoxicity studies.

The mouse carcinogenicity study and the rat combined toxicity/carcinogenicity study showed no treatment-related increase in tumor incidence. The Hazard Identification Assessment Review Committee classified phosalone is a **"not likely" human carcinogen**.

A microbial mutagenicity assay with *Salmonella typhimurium* did not result in mutagenic effects and there was no evidence of a clastogenic effect in an *in vitro* cytogenetic assay. In an *in vitro* unscheduled DNA synthesis (UDS) assay, phosalone was found to be weakly active for UDS induction.

In a rat metabolism study, phosalone was rapidly absorbed orally and extensively metabolized. The major route of elimination was urinary. Tissue residues were low with minimal potential for bioaccumulation.

B. Acute Toxicity

An acute oral LD_{50} study in rats and a delayed neurotoxicity in hens were evaluated by the HIARC. Phosalone was in acute toxicity category 2 by the oral route in rats. The hen test was negative for delayed neurotoxicity. Acute toxicity results are reported in Table 1 below.

Table 1. Acute Toxicity Profile of Phosalone.

GDLN	Study Type	MRID	Results	Tox Category
81-1	Acute Oral	00006716, 00006643	Male: 120-155 mg/kg Female: 90-135 mg/kg	II
81-7	Delayed Neurotoxicity, Hen	00137037 00137038	Negative for OPIDN	—

C. Subchronic Toxicity

Subchronic rat and dog feeding studies were not available. Requirements for these studies are satisfied by the acceptable chronic rat and dog feeding studies.

D. Chronic Toxicity

Chronic toxicity studies with phosalone included a chronic dog study and a combined chronic/carcinogenicity study in rats. Toxicity in both studies included body weight decrements and inhibition of plasma, red blood cell, and brain cholinesterase activity. A NOAEL (no-observed-adverse-effect level) for plasma cholinesterase inhibition was not determined in the chronic dog study, but the low dose was believed to be close to a NOAEL. Toxicity in the chronic rat study also included decreased testicular and adrenal weights and microscopic testicular, adrenal, and pituitary lesions.

Chronic Dog Study: In a chronic toxicity study in dogs (MRIDs 44792008 and 44792009), six beagle dogs/sex/group were administered Phosalone (94.5%) in the diet for one year at dose levels of 0, 5, 25 or 300 ppm (males: 0, 0.17, 0.89 or 11.15 mg/kg/day; females: 0, 0.19, 0.97 or 11.48 mg/kg/day). Two dogs were sacrificed during the study and were replaced. During Week 4, a 300 ppm male was sacrificed due to persistent anorexia and weight loss. Effects on clinical chemistry parameters and decreased cholinesterase levels were reported prior to death. There were no significant findings on gross or histopathology. It is likely that this death is treatment-related due to the elevated dose the animal received and observation of effects from the beginning of the study. The other animal which was replaced in Week 8 was a control animal which was sacrificed due to peritonitis subsequent to an inguinal hernia repair.

Three additional animals had inguinal hernias repaired during the study - 300 ppm male and 5 ppm female during Week 9 and a 300 ppm male at approximately Week 26. During Week 52, a 25 ppm male was sacrificed, rather than have the hernia repaired, since it was near the end of the study. Two animals (25 ppm males) required surgical treatment of wounds received during fighting.

There were sporadic episodes of vomiting and loose stools in all groups throughout the study. However, during Week 43, all dogs in the 300 ppm group vomited on one or more days, and had decreased food consumption and body weight. From Week 45 until termination, the majority of the dogs recovered.

There were no statistically significant differences in mean body weight, however all dogs receiving 300 ppm had weight losses, ranging from 0.1 to 1.6 kg, with 5 dogs having losses exceeding 1 kg during Week 43. The mean body weights for the 300 ppm males and females were decreased 18% and 12%, respectively, from the control value during Week 43. Although animals recovered during Week 45, the overall mean weight gain for both sexes was lower than the control values (% decrease: males: 11%; females: 8%). Decreased mean body weight gain in both the 25 and 300 ppm groups was evident during the first 13 weeks of the study. The decrease from control value was 18 and 25% for the 25 ppm males and females, respectively, and 35% and 30% for the 300 ppm males and females, respectively. For Weeks 0-52, the values for the 300 ppm males and females were decreased 50 and 26%, respectively. Mean food consumption was fairly consistent throughout the study, except during Week 43 when it was decreased 11% in males and 9% in females. Overall food consumption was statistically significantly decreased in the 300 ppm males, however this was only a 1% decrease from the control value.

There were no treatment-related effects on hematology and clinical chemistry parameters, except for cholinesterase (ChE). Plasma ChE was statistically significantly decreased at all time points (Weeks 2, 4, 13, 26 and 52) in the 25 ppm males (26-46%) and females (32-47%) and 300 ppm males (66-75%) and females (62-70%). Statistically significant decreases were also found in the 5 ppm males. Although the magnitude of the change (5-19%) was within the traditional limits of variability, a clear dose-response relationship was present. Erythrocyte (RBC) ChE was statistically significantly decreased in the 300 ppm males 30-76%) and females (40-72%) at the majority of time points. At Week 26, the 25 ppm males had a statistically significant decrease that was biologically significant (28%). Biologically significant values were seen at Week 52 for males (24% decrease) and females (34% decrease). Brain ChE was statistically significantly decreased in the 300 ppm group males (34%) and females (35%).

There were some changes in urinalysis parameters, however since there was no evidence of renal pathology on necropsy, these changes are not considered treatment-related. There were no treatment-related effects on organ weight and gross or histopathology findings.

The **systemic** no-observed-adverse-effect level (**NOAEL**) = 5 ppm in males (0.17 mg/kg/day) and females (0.19 mg/kg/day) and the systemic lowest-observed-adverse-effect level (**LOAEL**) = 25 ppm in males (0.89 mg/kg/day) and females (0.97 mg/kg/day) based on decreased body weight gain

The **cholinesterase** inhibition **NOAEL in males** could not be established based on statistically significant and dose-related decreases in plasma ChE activity in all dose groups. The cholinesterase **LOAEL in males** = 5 ppm (LDT = 0.17 mg/kg/day). The cholinesterase inhibition **LOAEL in females** = 25 ppm (0.097 mg/kg/day) based on decreased RBC and plasma ChE and the **NOAEL in females** = 5 ppm

This chronic toxicity study in the dogs is **acceptable/guideline** and **does satisfy** the guideline requirement for a chronic oral study (83-1; OPPTS 870.4100 in dogs.

Combined Carcinogenicity/Toxicity Study in Rats: In a chronic toxicity/carcinogenicity study (MRID 44801002), Phosalone (94.5% a.i.) was administered in the diet to 50 Crl:CD (SD) BR rats/sex/dose [main study] for 104 weeks at dose levels of 0 ppm, 5 ppm, 50 ppm, or 1000 ppm [lowered to 500 ppm from week 27 on]. Phosalone was administered to 15 rats/sex/group (control, low-, and mid-dose levels) or 25/sex (high dose) in a satellite study at the same dose levels for 52 weeks. These dose levels corresponded to 0.2, 1.8, and 20 mg/kg/day, respectively, in males; 0.2, 2.5, 31 mg/kg/day, respectively, in females. The dosing was considered adequate, based primarily on brain cholinesterase inhibition in both sexes.

Survival was not adversely affect; in fact, both sexes at the high-dose level had the highest survival rate, and the control rats of both sexes had the lowest. The high-dose females displayed an apparent, treatment-related, increase in the incidence of hyperactivity, which dissipated following the lowering of the dose at week 27. Both sexes at the high dose displayed decreased [males 89%-92%/females 77%-84% of control] body weight throughout the first year of the study. After the high-dose level was lowered at week 27, body weight was comparable among the groups. At study termination, comparable body weights were observed among the group [high-dose males 98%/high-dose females 96% of the control]. Body-weight gains during the 0-13 week interval were decreased for both sexes at the high-dose level [males 88%/females 65% of control]. Food consumption/efficiency and water consumption were also decreased for both sexes at the high-dose level, mainly during the first year of the study. No adverse, treatment-related, effects were observed on ophthalmoscopy, hematology, clinical chemistry [except cholinesterase activity], or urinalysis in either sex. There was a dose-related inhibition of both plasma and RBC cholinesterase activities in females at all time intervals measured. In males, inhibition of both plasma and RBC cholinesterase activities was observed mainly at the high-dose level throughout the study. Brain cholinesterase activity was inhibited at the high-dose level in both sexes at study termination.

There was no apparent treatment-related increase in tumor incidence in either sex compared with concurrent controls and historical controls.

The **NOAEL** 5 ppm **[0.2 mg/kg/day in males and females]**, and the **LOAEL** is 50 ppm **[1.8 mg/kg/day and 2.5 mg/kg/day in males and females, respectively]**, based on plasma and RBC cholinesterase inhibition [both sexes], decreased testes/epididymal weight [males], and an increased incidence of testicular lesions [tubular atrophy]. At the high-dose level, brain cholinesterase activity was decreased in both sexes [males 27% of control/females 40% of control], there was an increased incidence of hyperactivity [females], decreased adrenal weight [females], and increased incidences of pituitary hyperplasia [both sexes], vacuolated cortical cells of the adrenal [males], and aggregations of alveolar macrophages in the lungs [females]. There was no apparent increase in any tumor type that could be attributed to treatment.

This guideline chronic toxicity/carcinogenicity study is **acceptable/guideline**

[OPPTS 870.4300; §83-5], and **satisfies** the guideline requirement for a chronic toxicity/carcinogenicity study in rats.

E. Carcinogenicity

There was no evidence of a treatment-related increase in tumor incidence in either the mouse carcinogenicity study or the combined rat/carcinogenicity study. In accordance with the EPA *Proposed Guidelines for Carcinogen Risk Assessment* (April 10, 1996), the HIARC classified phosalone as a **"not likely"** human carcinogen.

Combined Carcinogenicity/Toxicity Study in Rats: See the chronic toxicity section of this document for the executive summary of the combined carcinogenicity/toxicity study in rats.

Mouse Carcinogenicity Study: In a mouse carcinogenicity study, (Accession 00065653), phosalone (95.3%) was administered for 2 years at dietary concentrations of 0, 15, 50, or 150 ppm (equivalent to 0, 2.25, 7.5, or 22 mg/kg/day) to 65 mice (control and high-dose) or 60 mice (low- and mid-dose) per sex per dose group. There was an interim sacrifice of 5 mice/sex from the control and high-dose groups at 6 weeks for cholinesterase determinations. Cholinesterase activity was also determined at termination for 5 mice/sex from the control and high-dose groups only.

Mortality was unaffected by treatment; survival ranged from 40-57% in the different dose groups. There was no effect of treatment upon body weight, weight gain, clinical signs, food consumption, or gross or microscopic pathology. Absolute and relative adrenal weights were increased in all treatment groups, however, this is not believed to be toxicologically significant in the absence of histological correlates.

Animals were fasted for 12 hours prior to cholinesterase determinations at 6 weeks, but not at termination. **Brain cholinesterase** activity was unaffected by treatment. **Plasma cholinesterase** activity in the high-dose group was decreased at week 6 in males (-55%) and females (-48%) and at week 105 in males (-81%) and females (-80%) in comparison to controls. **Red blood cell cholinesterase** activity was decreased in the high-dose group at week 6 in males (-19%) and females (-29%) and at week 105 in males (-61%) and females (-24%) in comparison to controls.

Uterine leiomyomas were increased (1/60, 1/60, 2/60, 3/60) as were leiomyosarcomas (1/60, 2/60, 2/60, 4/60) in the respective dose groups. These incidences were not statistically significant and were within the historical control ranges from the performing laboratory from 1977-1981 (uterine leiomyomas ranged from 0-10% and uterine leiomyosarcomas ranged from 0-6.78%, MRID 44792011).

In this study, the **LOAEL** was 150 ppm (highest dose tested, 22 mg/kg/day) based upon inhibition of plasma and red blood cell cholinesterase activity. A **NOAEL** was not determined because cholinesterase activity was not determined in low- and mid-dose groups. The LOAEL may have been lower than 150 ppm had cholinesterase activity been determined in low- and mid-dose groups. Dosing was judged adequate for a

carcinogenicity study based upon cholinesterase inhibition, although the animals could have tolerated a larger dose. This study is classified **acceptable/guideline**.

F. Developmental Toxicity

No developmental abnormalities occurred in acceptable developmental toxicity studies in rats and rabbits with phosalone. Developmental toxicity in both studies included post-implantation loss due to embryonic resorptions. Pregnancy rates and other developmental parameters were similar to control values. Severe maternal clinical signs related to cholinesterase inhibition occurred in both the rat developmental study (continuous chewing motions, hypersensitivity to noise, piloerection, dyspnea) and the rabbit developmental study (dyspnea, abdominal cramps, extension spasms, convulsions, lying prostrate). Cholinesterase activity was not determined in either study.

Developmental Toxicity in Rats: In a developmental toxicity study (MRID 41085001), 25 Wistar/HAN rats/dose group were given nominal doses of 0, 2, 10, or 20 mg/kg/day phosalone (93.6%) in 4% carboxymethylcellulose from gestation days 6-15. Corrected for analytical concentrations, doses were 0, 1.7, 8.6, or 16.6 mg/kg/day. Dams were euthanized on day 21 and uteri, ovaries, and fetuses examined. Cholinesterase measurements were not made.

No deaths occurred in the study. Clinical signs were limited to the high-dose group and continued during the post-treatment period. All animals in this group experienced continuous chewing motions, hypersensitivity to noise, piloerection, and dyspnea. Body weights were similar in all dose groups. Body weight gain in the high-dose group was decreased in comparison to controls during the treatment period (-34%) as was food consumption (-10%).

Pregnancy rates and mean numbers of corporae lutea and implants were similar among groups. There was an increased number of resorptions in the high-dose group (32 vs 17 in controls) with a corresponding increase in post-implantation loss (10% vs 5.1% in controls) and a slight decrease in the number of live fetuses/dam (12.0 vs 12.7 in controls).

The **NOAEL** for **maternal** toxicity is 8.6 mg/kg/day and the maternal LOAEL is 16.6 mg/kg/day based upon clinical signs and decreased body weight gain. The **NOAEL** for **developmental** toxicity is 8.6 mg/kg/day and the LOAEL is 16.6 mg/kg/day based on increased resorptions and post-implantation loss. This study is classified **acceptable/guideline** and satisfies requirements for developmental toxicity study in rats.

Developmental Toxicity in Rabbits: In a developmental toxicity study (MRID 41089501) 4 groups of pregnant Chinchilla Kfm: CHIN, Hybrids, SPF Qualified rabbits (16/dose) were given phosalone technical (93.5% a.i.) in 4% CMC (by gavage) at dose levels of 0, 1, 10, and 20 mg/kg/day from days 6 through 18 of gestation. The test article was administered at a volume 4 ml/kg body weight. Observations for maternal toxicity or mortality were conducted twice a day from GD 0 - 28. On GD 28, the dams were

euthanized and their uteri and ovaries were examined. The fetuses were examined both viscerally and externally. Cholinesterase activity was **not** measured in this study. A statistically significant decrease ($p \leq 0.05$) in food consumption, during the dosing period only, was observed in high-dose (20 mg/kg/day) group animals. A slight but not statistically significant overall weight loss and a decrease in body weight gain were reported for animals in this dose group. Animals in the high-dose group also exhibited evidence of dyspnea (12/16 animals), abdominal cramps (8/16 animals), and extension spasms and/or convulsions and/or lying prostrate (5/16 animals).

Under the conditions of this study, the **maternal LOAEL** is established at **20 mg/kg/day** (HDT) based on signs of toxicity (dyspnea, abdominal cramps, convulsions, etc.) and a statistically significant decrease in food consumption. The **maternal NOAEL** is **10 mg/kg/day**.

Developmental Toxicity - While the pregnancy rates were not affected by treatment with the test article, the statistically significant increased incidence of post-implantation losses reported at the 10 and 20 mg/kg/day dose levels ($p \leq 0.05$ and $p \leq 0.01$, respectively) suggest a compound-related effect. Total embryonic resorption was also noted for animals in these groups (2/16 at the high-dose and 1/16 at the mid-dose) while none was seen in the control. Mean fetal body weight did not appear to be affected by treatment with the test article. Under the conditions of this study, the **developmental LOAEL** is established at **10 mg/kg/day** based on post-implantation losses. The **developmental NOAEL** is established at **1 mg/kg/day**.

This study in the rabbit is **acceptable/guideline** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3) in rabbits.

G. Reproductive Toxicity

Phosalone treatment did not result in changes in reproductive parameters. Offspring toxicity included body weight decrements and pup mortality between birth and day 4 *post partum* at the high dose. Parental toxicity included plasma and red blood cell cholinesterase inhibition. A NOAEL for parental plasma cholinesterase inhibition was not determined, however the low dose was believed to be close to a NOAEL. No clinical signs were observed in the parents of either generation.

Reproductive Toxicity in Rats: In a 2-generation reproduction study [MRID 44792013], Phosalone [94.5% a.i.] was administered to 32 [F0]/28 [F1] Crl:CD(SD)VAF/Plus BR rats/sex/dose via the diet at dose levels of 0, 10, 50, and 400 ppm [F0 males 0.71, 3.56, 29.39 /females 0.78, 3.94, 32.82 mg/kg/day; F1 males 0.8, 3.98, 33.58/females 0.86, 4.3, 36.74 mg/kg/day, respectively] during the pre-mating period of 10 weeks [F0 generation]/16 weeks [F1 generation]. There was one litter per generation.

There were no treatment-related deaths of the parental rats in either generation. There were no adverse, treatment-related, effects on body weight, body-weight gain, or food consumption in either sex or generation during the dosing period, although at the

high-dose level, both sexes of the F1 offspring displayed a significantly lower body weight [89% of control] at the start of dosing at week 4. A slight decrease in water consumption was observed for both sexes, mainly at the high-dose level, in both generations. For the dams, although comparable body weights were observed among the F0-generation dams throughout gestation and lactation, body-weight gains were decreased throughout gestation [87%-93% of control] and lactation [69%-70% of control] at the high-dose level. For the F1-generation dams, slightly lower body weights [93%-95% of control] were observed at the high-dose level, mainly during lactation, and body-weight gains were decreased [80%-82% of control] also, mainly during lactation.

Pregnancy rates were comparable among the groups in both generations, and the majority of the females mated within the first four days after pairing. In both generations, there were no significant differences among the groups with respect to the duration of gestation, the number of implantation sites, implantation losses [%], or sex ratios, and the majority of the dams delivered live fetuses.

There was a dose-related decrease in plasma and erythrocyte cholinesterase activity in both sexes and in both generations at study termination, and the magnitude of the decreases was similar for both generations. The decrease in RBC cholinesterase activity occurred at all dose levels in the F0 males and the F1 females. Although brain tissue samples were collected for brain cholinesterase activity measurements, the analyses were not performed. There were no apparent, treatment-related, effects on organ weights, and gross and microscopic findings were comparable among the groups for both sexes and both generations.

There was an increase in pup death between birth and day 4 *post partum* of the offspring of both generations, although the effect was greater in the first generation. At birth, body weights of the F1 offspring were comparable among the groups, but by day 4 *post partum*, body weight was significantly reduced [91% of control] at the high-dose level compared to the control and remained lower throughout lactation [90% of control]. Slightly lower body weights [95% of control] were observed at birth of the F2 pups at the high-dose level, and decreased body weights [84%-87% of control] were observed throughout lactation at the high-dose level, with the magnitude of the deficit increasing with time. Similarly, litter weights were comparable at birth in both generations, but by day 4 and throughout lactation, decreased litter weights were observed at the high-dose level in both generations. There was a marginal delay at the high-dose level in surface righting and startle response in the F1 weanlings and a marginal delay in male post-weaning development [balanopreputial cleavage]. There were no apparent adverse effects observed on pups at either the mid- or low-dose level.

No **NOAEL for maternal/paternal toxicity** was determined, based on decreased erythrocyte [RBC] cholinesterase activity at all dose levels in F0 males and F1 females at 10 ppm [F0 males 0.7/F0 females 0.8 mg/kg/day; F1 males 0.8/F1 females 0.9 mg/kg/day], which is the **LOAEL**. Decreased plasma and/or RBC cholinesterase activities in both sexes was observed at 50 ppm [F0 males 3.6/F0 females 3.9 mg/kg/day; F1 males 4.0/F1 females 4.3 mg/kg/day]. The **reproductive NOAEL** is 400 ppm [F0 males 29.4/F0 females 32.8 mg/kg/day; F1 males 33.6/F1 females 36.7 mg/kg/day], the highest dose

tested. The **neonatal NOAEL** is 50 ppm [F0 males 3.6/F0 females 3.9 mg/kg/day; F1 males 4.0/F1 females 4.3 mg/kg/day], and the **LOAEL** is 400 ppm [F0 males 29.4/F0 females 32.8 mg/kg/day; F1 males 33.6/F1 females 36.7 mg/kg/day], based on increased mortality during the day 0-4 *post partum* interval in both generations, and decreased mean pup body weight and litter weight.

This guideline [§83-4; OPPTS 870.3800] 2-generation reproduction study in rats is classified **acceptable/guideline**.

H. Mutagenicity Studies

The pre-1991 mutagenicity initial testing battery guidelines are satisfied by the battery of mutagenicity studies. There was no evidence of a clastogenic effect in an *in vitro* cytogenetic assay. A microbial mutagenicity assay with *Salmonella typhimurium* did not result in mutagenic effects. In an *in vitro* unscheduled DNA synthesis (UDS) assay, phosalone was found to be weakly active for UDS induction. **It is recommended that the UDS assay be repeated to confirm or refute questionable results in this study.**

Cytogenetic Assay: In an *in vitro* cytogenetic assay (MRID No. 41143301), Chinese hamster ovary (CHO) cells were exposed to Phosalone technical (94.35%) at concentrations of 15.0-200 μg/mL without S9 activation and 37.5-300 μg/mL with S9 activation. Cells were fixed 20 hours after treatment and examined for the frequency of structural chromosome aberrations. Severe cytotoxicity was evident at 200 μg/mL -S9 and 300 μg/mL +S9. The positive controls induced the expected high yield of cells with aberrant chromosome morphology. There was, however, **no evidence of a clastogenic effect** induced by Phosalone technical in either the presence or absence of S9 activation. This study is classified **acceptable/guideline** and satisfies the guideline requirements for an *in vitro* mammalian cell cytogenetic assay.

Bacterial Mutagenicity Assay: In independent microbial mutagenicity assays (MRID No. 447920115), Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 were exposed to 250-10,000 µg/plate Phosalone technical (94%) in the initial and confirmatory trials both in the presence and absence of S9 activation. A repeat trial was also conducted with strains TA1538 and TA98 using comparable nonactivated and S9-activated doses. Compound precipitation was reported at doses $\geq$1000 µg/plate+/-S9. There was no evidence of cytotoxicity at any dose. All strains responded in the expected manner to the appropriate positive control. There was also **no indication that Phosalone technical induced a mutagenic effect at any dose with or without S9 activation**. The study is classified **acceptable/guideline** and satisfies the guideline requirements for a bacterial gene mutation assay.

Unscheduled DNA Synthesis Assay: In an *in vitro* unscheduled DNA synthesis (UDS) assay (MRID No. 44792016), primary rat hepatocytes recovered from a male Fischer-344 rat were exposed to 15 doses (0.005-252 µg/mL) Phosalone technical (94%)

for 18.4 hours. Recovered hepatocytes treated with 0.503-25.2 µg/mL were scored for UDS. Severe cytotoxicity was seen at >25.2 µg/mL and moderate cytotoxicity (64.7% survival) was observed at 25.2 µg/mL. The positive control induced the expected high yield of hepatocytes with net nuclear grains. There was, however, an increase in the percentage of cells in repair (18% versus 2% in the solvent control) at 25.5 µg/mL; the increase in net nuclear grain counts at this dose (2.1 versus -1.09 in the solvent control) did not exceed the criterion used to indicate UDS activity. The response was limited to this dose with no evidence of UDS at lower levels (0.503-10.1 µg/mL). Based on these findings, Phosalone technical is considered to be **weakly active for UDS induction** in this *in vitro* test system. **It is recommended that the UDS assay be repeated to confirm or refute the findings of this study.** The study is classified **acceptable/guideline** and satisfies the requirements for FIFRA Test Guideline 84-2 for a UDS assay.

I. Metabolism Study

Phosalone was rapidly absorbed orally and extensively metabolized. The major route of elimination was urinary. High-dose females had a slower rate of elimination than males. Tissue residues were low with minimal potential for bioaccumulation. The majority of metabolites in urine were not identified and this study is classified **unacceptable/guideline but upgradable** if urinary metabolites are identified or if an explanation is offered as to why identification is not possible.

Metabolism Study in Rats: In a rat metabolism study (MRID No. 44792017), Crl:CD (SD) BR rats (5 animals/sex/group) received a single gavage dose of ^{14}C-ring-labeled phosalone at 1 or 50 mg/kg or repeated gavage doses (14 daily doses) of unlabeled phosalone at 1 mg/kg followed by a single gavage dose of ^{14}C-phosalone at 1 mg/kg.

The single low, multiple low, and high oral dose studies indicate that phosalone is rapidly absorbed and extensively metabolized. In general, the majority of the radioactivity was eliminated in the urine and feces within 24 hours after dosing for all animals except for the high dose females. The major route of elimination was urinary (approximately 62-70% of the dose) over a 3-day period. No marked sex-related difference was observed in the elimination patterns. The pattern of elimination are similar with either single or repeated dosing. The tissues, cage wash and expired air as CO_2 contained 0.1-1.3%,11.4-16.2% and up to 0.1% of the administered dose, respectively.

In all manners of dosing, tissue residues were very low indicating that the potential for bioaccumulation of phosalone is minimal.

For the 1 mg/kg group (single dose), **peak blood and plasma concentrations** were reached in males at 1 hour and females at 0.5-2 hours. Residue levels in blood and plasma declined steadily to low level by 24 hours. For the 50 mg/kg group, peak blood concentration was reached in males at 3-6 hours and in females at 3 and 24 hours. In these animals, peak plasma concentration was reached in males at 6 hours and in females at 3 hours and between 15 and 24 hours. Residue levels in blood and plasma declined steadily and they were non-detectable at 168 hours post-dose. The data indicated that in

high dose females, a slower elimination of radioactivity was found.

In the urine of rats administered with 50 mg/kg or 1 mg/kg (single dose), there were at least 8 **metabolites of phosalone**, however, the majority of the radioactivity (57-62% of dose) was unidentified. These unknown metabolites were more polar than the sulfoxide of phosalone (RP 19889; up to 1% of the dose) and the sulfone of phosalone (RP 19888; up to 0.2%). The amount of the parent in urine was less than 1% of the administered dose. In the feces, there were at least 14 metabolites of phosalone mostly in low amounts and the only metabolite identified was the sulfide of phosalone (RP 19914; 0.4-0.9% of the dose). The amount of the parent accounted for 12-16% of the administered dose.

This metabolism study in the rat is classified **unacceptable/guideline but upgradable** and does not satisfy the guideline requirement for a metabolism study (85-1) in rat because the majority of the radioactivity in urine, accounting for 57.4-62.9% of the administered dose, was not identified. If identification is not possible, a justification/explanation should be provided in the final report. However, the data provide reasonable understanding with respect to absorption, distribution, elimination, and peak blood and plasma concentration of this chemical.

J. Neurotoxicity Studies

Neurotoxicity studies with phosalone included acute, subchronic, acute rangefinding, and time-of-peak effect studies in rats and an acute delayed neurotoxicity study in hens. Neurotoxic effects in rats included plasma, red blood cell, and brain cholinesterase inhibition and clinical signs related to cholinesterase inhibition. The time of peak effect was determined to be 6 hours after treatment. Plasma cholinesterase inhibition occurred at the first measurement period of 2.5 hours and clinical signs were mainly observed at the 6^{th} and 7^{th} hour post-treatment. The acute hen study was negative for delayed neuropathy and a subchronic study in hens was not required.

Acute Neurotoxicity Study in Rats: In an acute oral neurotoxicity study (MRID 44852503) 4 groups of 28 - 35 days old Crl: CD BR rats were given a single oral dose (by gavage) of phosalone technical (93.8% a.i.), in corn oil at doses of 0, 10, 25, or 60 mg/kg of body weight. The study consisted of two sets of experiments: 1) the main study groups contained 10 rats/sex/dose and were used for neurobehavioral testing and clinical signs observations; 2) the satellite group contained 5 rats/sex/dose and were used for determination of cholinesterase activity. All animals were observed daily for signs of toxicity and twice a day for signs of moribundity and/or mortality.

Clinical signs such as brown nasal staining, damp urogenital area, loose feces, and wet lower jaw fur were first reported at the 25 mg/kg dose level approximately 3 - 4 hours after exposure to the test article (3/10 females and 1/10 males in the main group; and 4/8 females and 3/5 males in the satellite group).

At the 60 mg/kg dose level, 20/20 females (10 main group, 10 satellite group) and 12/15 males (7/10 main group, 5/5 satellite group) showed a myriad of clinical signs

including tremors, hunched posture, clonic jaws, unsteady gait, cold extremities, exophthalmia, and wet/damp urogenital/anogenital region approximately 3 - 4 hours after exposure to the test substance. With the exception of fur staining, all clinical signs of toxicity had resolved by the 24 hour examination period. No mortalities were reported at any dose level during the study period. A slight but statistically significant decrease in body weight gain was noted for males at the 60 mg/kg dose level during the first week of the study. A concomitant decrease in food consumption (not statistically significant) was also noted for this time period.

The first indications of compound-related effects detected by the Functional Operational Battery (FOB) were reported in both males and females at the 60 mg/kg dose during the 6 hour post-dosing observation period and included tremors, exophthalmia, clonic jaws, piloerection, unusual gait (walking on toes), hypothermia, decreased activity, and wet anogenital region. These symptoms resolved within the first week after treatment with the test article and were no longer evident at the Day 8 observation period.

A statistically significant inhibition of plasma cholinesterase activity (PChE) was reported at all dose levels six hours after exposure to the test article (time of peak effect). This inhibition persisted at the 60 and 25 mg/kg (males only) dose levels for 24 hours but was no longer evident at the Day 7 observation period. Additionally, a statistically significant RBC ChE inhibition, which persisted during the first 24 hours of the study period, was noted at the 60 mg/kg dose level. Animals in the high-dose group (60 mg/kg) showed evidence of statistically significant plasma ChE inhibition at the Day 15 examination (end of the study period).

No compound-related abnormalities were found in histological examination. Under the conditions of this study, the **LOAEL** is established at **10 mg/kg** based on plasma cholinesterase inhibition in males and females. No clear **NOAEL** could be established for this study. This study is classified **acceptable/guideline** and satisfies the guideline requirement for an acute neurotoxicity study (OPPTS 870.6200; §81-8) in rats.

Acute Rangefinding Neurotoxicity Study: Doses for the acute neurotoxicity study were based upon a range finding study. In the range finding study (MRID 44852502) Crl: CD BR rats were exposed to the test article once (by gavage) at doses of 12, 60, and 80 mg/kg.

Animals in the 12 mg/kg dose group did not exhibit evidence of any compound-related effects during FOB testing. Though animals in the 60 mg/kg group (1/sex) were not subjected to FOB testing, numerous signs of toxicity such as tremors, piloerection, and excessive chewing were seen at the 4 hour observation period. By the 5 and 6 hour observation periods the range of signs of toxicity had increased to include exophthalmia, hypothermia, hunched posture, and excessive salivation.

FOB testing animals in the 80 mg/kg dose group (3/sex), revealed signs of toxicity at the 5 hour observation period. These signs comprised of tremors, decreased body temperature, and exophthalmia (females only), as well as excessive chewing motion in both sexes. Six hours post-dosing, tremors and decreased body temperature were also reported for males. Males had completely recovered by the 24 hour period while signs of

toxicity persisted in females. One female had to be humanely sacrificed 3 days post-dosing due to distress. The 80 mg/kg dose was considered to be too high for further studies and the 60 mg/kg dose was selected as the HDT (highest dose tested) for further studies.

Time of Peak Effect Neurotoxicity Study: In the time of peak effect study (MRID 44852501), 10 Crl: CD BR rats/sex were subjected to a single exposure (by gavage) to phosalone technical at a dose of 60 mg/kg to determine the time of peak effect. The animals were observed for evidence of signs of toxicity 4, 5, 6, 7, and 8 hours post-dosing and daily thereafter until the termination of the study (Day 8).

Numerous signs of toxicity (excessive salivation, tremors, hunched posture, unsteady gait, hypothermia, piloerection, wet urogenital region and labored breathing) were evident shortly after dosing with a preponderance of the effects being observed at the 6 and 7 hour observation period. Although males had completely recovered by Day 2 of the study, signs of toxicity were still apparent in females.

Plasma and erythrocyte cholinesterase activity (PChE and RChE) were measured prior to dosing, and at 2.5, 4, 6, 8, and 24 hours after administration of the test substance while brain cholinesterase activity was measured on day 8 of the study. Statistically significant ($p \leq 0.01$) PChE inhibition (both sexes) was first seen 2.5 hours after exposure to the test article and persisted until the 24 hour measurement period. RChE inhibition was first reported at the 4 hour observation period, however, the pattern of inhibition over observation time points was not as consistent as that of PChE. Measurements of brain cholinesterase activity at the end of the study period (Day 8) revealed a statistically significant level of BChE inhibition for both males and females.

Given the extent of clinical signs seen at different time points, the time of peak effect was established to be 6 hours after exposure to the test article.

Subchronic Neurotoxicity: In a subchronic neurotoxicity study (MRID 44852504), 10 Crl:CD BR rats/sex/dose group received dietary concentrations of 0, 50, 150, or 600 ppm phosalone (93.8%) in the diet for 13 or 14 weeks. Dietary concentrations were equivalent to 0, 3.9, 11.5, or 45.9 mg/kg/day for males and 0, 4.4, 12.6, or 56.0 mg/kg/day for females. Rats were given functional observational battery and motor activity tests pre-dose, and at 4, 8, and 13 weeks. After 13 weeks of treatment, rat were sacrificed and 5 rats/sex/dose group were perfused for microscopic examination of the nervous system and 5 rats/sex/dose group had plasma, red blood cell (RBC), and brain cholinesterase (ChE) activity tested by the Ellman method. An additional 10 rats/sex/group comprised a satellite group from which 5 rats/sex/dose were sacrificed at 4 and 8 weeks for brain ChE measurements. RBC and plasma ChE were also determined in this group at pretest and at weeks 4 and 8.

No deaths or clinical signs occurred in the study. Females in the high-dose group had reduced body weights (-10% to -14% of controls) throughout the study. Body weight gain in high-dose females after 13 weeks of treatment was reduced in comparison to controls (-28%). Body weights and weight gains in other groups were comparable to controls. Food consumption and food efficiency were generally comparable among the

different groups. Forelimb and hindlimb grip strength were decreased in high-dose males and/or females at various time intervals. Landing footsplay values were decreased in high-dose males and in all female treatment groups in several time intervals. Also reported were increased incidence of hairloss and "badly groomed" in high-dose females at week 13.

Plasma cholinesterase (ChE) was inhibited in mid- and high-dose males and in all female treatment groups. Red blood cell ChE was inhibited in mid-dose and high-dose males and in all female treatment groups. Brain ChE was inhibited in all male treatment groups and in mid- and high-dose females.

The **LOAEL** is 50 ppm, the lowest dose tested **(males: 3.9 mg/kg/day; females: 4.4 mg/kg/day)**, based upon inhibition of brain ChE activity in males and inhibition of plasma and RBC ChE activity in females; a **NOAEL** was not established. This study is classified **acceptable/guideline**.

II. FOOD QUALITY PROTECTION ACT ISSUES

A. Susceptibility Issues

The data provided no indication of increased susceptibility in rats from *in utero* and/or post natal exposure to phosalone. In the prenatal developmental toxicity study in rats and the two-generation reproduction study in rats, effects in the fetuses and offspring were observed at doses higher than those producing maternal or parental effects.

In the prenatal developmental toxicity studies in rabbits, the developmental NOAEL (1 mg/kg/day) was lower than the maternal NOAEL (10 mg/kg/day) indicating an apparent quantitative increase in fetal sensitivity in rabbits following *in utero* exposures to phosalone. However, this was not considered a true quantitative increase in fetal sensitivity because (1) the endpoint was not a litter effect and (2) cholinesterase activity was not determined in this study and based upon information from other studies it is believed that cholinesterase inhibition was occurring at lower doses than the reported maternal endpoint. Therefore, it was considered unlikely that there was a true quantitative increase in fetal sensitivity. (See the HIARC Document, August 12, 1999, for more details.)

B. Safety Factor Recommendation and Rationale

The Food Quality Protection Act (FQPA) Safety Factor Committee met on August 16, 1999, to evaluate the need for the 10x FQPA safety factor for protection of infants and children. The FQPA Safety Factor Committee recommended that the **10x FQPA safety factor** for protection of infants and children be **reduced to 1x.** The Committee concluded that the safety factor could be removed for phosalone because: (1) the toxicology database is complete for FQPA assessment; (2) toxicity data provided no indication of qualitative or quantitative increased susceptibility of young rats or rabbits to phosalone;

(3) adequate actual data, surrogate data, and/or modeling outputs were available to satisfactorily assess dietary exposure (no drinking water or residential assessment is required for phosalone).

III. TOXICITY ENDPOINT SELECTION

The Hazard Identification Assessment Review Committee (HIARC) evaluated the toxicological database for phosalone on July 22, 1999 and selected toxicity endpoints for dietary exposure (HIARC Document 013710, August 12, 1999). The endpoints and doses are summarized in Table 2.

The chronic reference dose (RfD or cRfD) is an estimate of the level of daily dietary exposure to a pesticide residue which, over a 70-year human life span, is believed to have no significant deleterious effects. The acute reference dose (aRfD) is an estimate of the level of one-day dietary exposure to a pesticide residue which is believed to have no significant deleterious effects. Acute and chronic RfDs are determined by dividing the no-observed-adverse-effect level (NOAEL) from the selected study by uncertainty factors.

The population adjusted dose (PAD) is new terminology and refers to an RfD which has been adjusted to take into account the FQPA safety factor. The PAD is determined by dividing the RfD by the FQPA safety factor. For phosalone, the FQPA safety factor = 1, and the acute and chronic RfDs are equivalent to the acute and chronic PADs, respectively.

The aPAD for the general population (including infants and children) is 0.03 mg/kg/day. This endpoint is from an acute neurotoxicity study in rats with a LOAEL of 10 mg/kg/day, the lowest dose tested. Although a NOAEL for plasma cholinesterase was not determined in this study, the LOAEL is believed to be close to a NOAEL. Uncertainty factors total 300x (10x for interspecies extrapolation, 10x for intraspecies variation, and 3x for lack of a NOAEL). Plasma ChE inhibition is a sensitive endpoint for phosalone.

The acute population adjusted dose (aPAD) for the subpopulation of females 13 years of age or greater is 0.01 mg/kg/day. This endpoint is from a developmental study in rabbits with a no-observed-adverse-effect level (NOAEL) of 1.0 mg/kg/day and a lowest-observed-adverse-effect level (LOAEL) of 10 mg/kg/day based upon post implantation loss. The uncertainty factors total 100x (10x for interspecies extrapolation and 10x for intraspecies variation). An increase in fetal resorptions is an effect which could occur after a single exposure. The selected endpoint is a conservative indicator of toxicity because it was based on total resorptions and was not a litter effect.

The chronic PAD (for all population subgroups) is 0.002 mg/kg/day. This endpoint is from a 2-year rat feeding study with a NOAEL of 0.2 mg/kg/day and a LOAEL of 1.8 mg/kg/day based on plasma and red blood cell cholinesterase inhibition in both sexes and decreased testes/epididymal weight and an increased incidence of testicular lesions in males. The uncertainty factors total 100x (10x for interspecies extrapolation and 10x for intraspecies variation).

TABLE 2. Toxicological endpoints for use in human risk assessment

EXPOSURE	DOSE (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary (General population including infants and children)	LOAEL = 10	Plasma ChE inhibition	Acute neurotoxicity in rats
	UF =300	**Acute PAD = 0.03 mg/kg /day**	
Acute Dietary (Females 13+)	Developmental NOAEL = 1	Post-implantation loss	Developmental toxicity in rabbits
	UF =100	**Acute PAD= 0.01 mg/kg /day**	
Chronic Dietary	NOAEL = 0.2	Plasma and RBC ChE inhibition (both sexes), decreased testicular weight and lesions	2-Year Rat Study
	UF =100	**Chronic PAD = 0.002 mg/kg/day**	
Dermal or Inhalation Endpoints	---	None selected. The phosalone reregistration action is for an import tolerance.	—

IV. DATA GAPS

The database is complete with the exception of a required unscheduled DNA synthesis (UDS) assay. The UDS assay is required to confirm questionable results in the submitted UDS assay. The metabolism study was classified unacceptable/guideline but upgradable because the majority of the radioactivity in urine was not identified. If identification of the metabolites is not possible, a justification/explanation should be provided in the final report.